Brain, Behavior, and Immunity 57 (2016) 10–20

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Brain injury induces specific changes in the caecal microbiota of mice via altered autonomic activity and mucoprotein production

A. Houlden [a], M. Goldrick [a], D. Brough [a], E.S. Vizi [c,d], N. Lénárt [b], B. Martinecz [b], I.S. Roberts [a,*], A. Denes [a,b,*]

[a] Faculty of Life Sciences, University of Manchester, Manchester, UK
[b] Laboratory of Neuroimmunology, Institute of Experimental Medicine, Budapest, Hungary
[c] Laboratory of Drug Research, Institute of Experimental Medicine, Hungarian Academy of Sciences, P.O.B. 67, H-1450 Budapest, Hungary
[d] Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

ARTICLE INFO

Article history:
Received 13 January 2016
Received in revised form 9 March 2016
Accepted 5 April 2016
Available online 6 April 2016

Keywords:
Stroke
Microbiota
Cerebral ischemia
Inflammation
Noradrenaline
Gut
Mucoprotein

ABSTRACT

Intestinal microbiota are critical for health with changes associated with diverse human diseases. Research suggests that altered intestinal microbiota can profoundly affect brain function. However, whether altering brain function directly affects the microbiota is unknown. Since it is currently unclear how brain injury induces clinical complications such as infections or paralytic ileus, key contributors to prolonged hospitalization and death post-stroke, we tested in mice the hypothesis that brain damage induced changes in the intestinal microbiota. Experimental stroke altered the composition of caecal microbiota, with specific changes in Peptococcaceae and Prevotellaceae correlating with the extent of injury. These effects are mediated by noradrenaline release from the autonomic nervous system with altered caecal mucoprotein production and goblet cell numbers. Traumatic brain injury also caused changes in the gut microbiota, confirming brain injury effects gut microbiota. Changes in intestinal microbiota after brain injury may affect recovery and treatment of patients should appreciate such changes.

1. Introduction

It is becoming clear that intestinal microbiota play key roles in both host development and in maintaining homeostasis. Intestinal microbiota change with age and are also influenced by environmental factors such as diet, and disease (Flint et al., 2012; Wu, 2011; Spor et al., 2011). Recent research highlights the key role of microbial communities in the large intestine in essential immune defence mechanisms and control of inflammatory responses (Abt et al., 2012; Ganal et al., 2012). Impaired regulation of the intestinal microbiota is also known to contribute to diseases of the intestinal tract, and is linked with the development of diverse inflammatory conditions such as sepsis, metabolic disease or cancer (Ayres et al., 2012; Dupont and Dupont, 2011; Le Chatelier et al., 2013; Yoshimoto et al., 2013). Altered intestinal microbiota have also recently been linked to neuro-behavioural problems such as autism (Hsiao et al., 2013; Kang et al., 2013). In fact the effect of microbiota on brain function is profound with microbiota known to influence brain specific activity such as anxiety like behaviour, learning and memory, microglial activity and blood brain barrier integrity (Luczynski, 2016). However, much less is known about whether changes in the intestinal microbiota are themselves influenced by central nervous system function. Brain injury caused by stroke is the most common cause of lasting disability worldwide and has a huge socio-economic impact. Beyond the detrimental effects of the initial injury on outcome after acute cerebrovascular events, one of the key causes for death or prolonged hospitalization and impaired recovery of patients is the development of post-stroke infections (Dirnagl et al., 2007). In some clinical studies, preventive antibiotic therapy was found beneficial (Hetze et al., 2013), and recent data indicate that pattern recognition receptors that can recognise microbiota-derived products could contribute to stroke outcome (Denes et al., 2015; Caso et al., 2008). The enteric nervous system is under central autonomic control and it is believed that the autonomic nervous system contributes to regulation of intestinal immunity and microbiota (de Jonge, 2013). We and others have shown that acute brain injury induces diverse autonomic, neuroendocrine and inflammatory changes, which manifest in several organs in the body, leading to immunosuppression and the development of

* Corresponding authors at: Faculty of Life Sciences, University of Manchester, Manchester, UK (I.S. Roberts); Laboratory of Neuroimmunology, Institute of Experimental Medicine, Budapest, Hungary (A. Denes).
E-mail addresses: i.s.roberts@manchester.ac.uk (I.S. Roberts), denesa@koki.hu (A. Denes).

http://dx.doi.org/10.1016/j.bbi.2016.04.003
0889-1591/

infectious complications (Dirnagl et al., 2007; Denes et al., 2010). The impact of an acute brain injury on the intestinal microbiota, and whether this is also influenced by neuroendocrine and inflammatory changes as a result of injury, is not known, but could be of importance to the outcome and recovery of the patient.

Here we tested the hypothesis that an acute brain injury induced by experimental stroke or traumatic brain injury would induce specific changes in the gut microbiota. We demonstrate that brain injury profoundly impacts on microbial communities in the caecum and that brain injury is associated with specific changes in microbiota. We propose that these changes are due to increased noradrenaline (NE) release from the autonomic nervous system into the gut. These changes may influence recovery after an acute brain injury.

2. Methods

2.1. Mice

Male 10–14 week-old C57BL/6 mice were kept at 22 °C ± 1 °C and 65% humidity with a 12 h light-dark cycle and had free access to food and water. All animal procedures were performed under appropriate project licence authority and adhered to the UK Animals (Scientific Procedures) Act (1986) and the Hungarian Act of Animal Care and Experimentation (1998; XXVIII, Section 243/1998), approved by the Animal Care and Use Committee of the IEM.

2.2. Middle cerebral artery occlusion (MCAo)

Transient focal cerebral ischemia was induced as described previously (Dénes et al., 2010). Briefly, mice were anaesthetised with isoflurane, the common carotid artery was exposed and cerebral ischemia was induced by an intraluminal filament that was advanced along the internal carotid artery, to occlude middle cerebral artery. After 60 min of MCAo, reperfusion was induced for 4 h or 72 h prior to sacrifice. Core body temperature was maintained at 37.0 °C ± 0.5 °C throughout the surgery by a heating blanket (Homeothermic Blanket Control Unit; Harvard Apparatus, Kent, UK) and monitored after recovery. After surgery, animals were returned to their cages and allowed free access to water and food. Neurological deficit in mice was assessed by using Bederson scores (4 point scale of increasing neurological deficit) as described previously (Dénes et al., 2010). Animals that showed no obvious neurological deficit at 4 h reperfusion after MCAo (score 1 at minimum) have been excluded from the studies pre hoc (n = 1).

2.3. Surgical controls

We investigated the effect of surgical manipulation and anaesthesia in the absence of experimental stroke on changes in the intestinal microbiota. To achieve this, two separate experimental conditions were used; the first involved only anaesthesia with no surgical manipulation, the second sham surgery, during which mice underwent all procedures as in the MCAo group, except for occlusion of the MCA with an intraluminal filament.

2.4. Traumatic brain injury (TBI)

A closed head model of TBI was performed in mice under isoflurane anaesthesia similarly to what has been described earlier, with slight modifications (Umschweif et al., 2014). After induction of anaesthesia, the skull was exposed by a small, midline longitudinal incision. The head was held in place and a plastic cone was placed on the skull 2 mm lateral of the midline after which a 100 g weight was allowed to fall on the top of the cone from a preestablished height resulting in injury to the left hemisphere, which localised around the affected cerebral cortex (Fig. 7). Mice were allowed to recover and 1 ml of saline was injected subcutaneously for rehydration. We used a relative mild form of TBI resulting in 2 out of 7 mice with substantial neurological deficit as assessed 72 h later. Sham animals were subjected to the same procedure except for head injury.

2.5. Pharmacological manipulation of the sympathetic nervous system

A group of mice was injected with the NE reuptake inhibitor atomoxetine (Sigma, 0.1 mg kg^{-1}) and the α2-adrenergic receptor antagonist yohimbine (Sigma, 1 mg kg^{-1}) administered intraperitoneally (0.2 ml/mouse in total), once daily for three subsequent days. Another group of mice received a single intraperitoneal injection of 6-hydroxydopamine (6-OHDA) (0.2 ml, Sigma, 100 mg kg^{-1}) followed by 0.2 ml sterile saline for two subsequent days. Control mice were administered 0.2 ml saline daily for three days. Mice were sacrificed 72 h after the first injection; the caecum was quickly removed and was kept at −80 °C until use.

2.6. ELISA

To measure inflammatory changes and neurotransmitter levels in the gut, caecum tissues were washed in sterile saline and homogenised as described previously (Dénes et al., 2010). Samples were kept at −20 °C until processing. Protein concentrations calculated using a BCA assay (Pierce/Themo Fisher Scientific). Caecum homogenates were measured for granulocyte-colony stimulating factor (G-CSF), RANTES (CCL5), KC (CXCL1), MMP-9, interleukin 6 (IL-6), ICAM-1, VCAM-1 (R & D Systems, UK), adrenaline and noradrenaline (Eagle Biosciences, NH, USA), substance P (R & D Systems, UK), and serotonin (Enzo, UK) according to the manufacturers protocol.

2.7. DNA extraction

Genomic DNA was extracted directly from total caecal content (~250 mg) using the QIAamp DNA Stool Mini Kit (Qiagen) with pathogen protocol.

2.8. Community profiling

Bacterial communities were profiled in the mouse caecum using Denaturing Gradient Gel Electrophoresis (DGGE), 454 sequencing (Roche, USA), and Illumina MiSeq (USA). DGGE assessment of the bacterial communities was as follows: PCR amplification of the 16S rRNA gene used universal primers 341F-GC and 518R (Muyzer et al., 1993), and reaction conditions: 5U BioTaq polymerase in 1X buffer (Bioline, UK), 1.5 mM $MgCl_2$, 20 pmol primers, 0.2 mM dNTPs, 5 μg BSA, and 10–50 ng of template DNA in a final volume of 50 μL. The cycle sequence consisted of initial denaturation step of 95 °C 5 min, then 30 cycles of 95 °C 1 min; 55 °C 1 min; 72 °C 1 min, and final extension of 72 °C 10 min. PCR products were purified (QiaGEN Minelute kit) before loading onto a DGGE gel (150 ng/lane). Samples were separated using the D-code system (Bio-Rad, USA) on 10% w/v acrylamide gel with a gradient of 30–70% denaturant at 60 °C for 16 h at 63 V. Gels were stained for 30 min using SYBR Gold (Invitrogen, USA). DGGE Gels were analysed with Phoretix 1D Advanced gel analysis software (Ver. 5.0, Nonlinear Dynamics Ltd.), with binary matrix of band presence/absence of individual bands used for sample comparison.

2.8.1. Pyrosequencing

454 sequencing of the bacterial communities were as follows: The 16S rRNA gene was amplified using the modified 16S primers 66f and 518R (italicised) to include Lib-A (underlined) linker